In claim 6, delete "chimeric" and insert therefor --interspecies--.

Please add the following new claim.

--44. The composition of claim 1, wherein the oligopeptide comprises at least two positively-charged amino acids.--

REMARKS

The Invention

Claims 1-21 are being examined in the current application. The invention is directed to compositions and methods for internally labeling a cell. The composition of claim 1 comprises (1) a ligand which specifically binds to a cell surface receptor and becomes internalized by the cell, (2) an oligopeptide which comprises at least one positively-charged amino acid residue and at least one D-amino acid residue; and (3) a label which is covalently bound to the oligopeptide. Dependent claims 2-21 specify the features of the ligand, the oligopeptide, and the label in various embodiments.

The Amendments

Claim 1 has been amended to clarify that the oligopeptide does not specifically bind to the surface antigen. This aspect of the claim is disclosed both explicitly and inherently in the specification. The specification teaches the use of certain oligopeptides as degradation-resistant means of attaching a label to a ligand. "It is a discovery of the present invention that conjugation of radiolabels to ligands that bind to cell surface antigens via positively-charged, proteolysis-resistant

oligopeptides improves the effectiveness of radioimmunotherapy." Specification at page 5, lines 2-4, emphasis added. The specification does not teach that the oligopeptide can serve as the ligand itself, and it does not disclose any embodiments in which the oligopeptide serves as the ligand. The specification teaches an oligopeptide which conjugates a label to a ligand. Claim 1 recites a ligand and an oligopeptide as separate components of a composition. Indeed, for most surface antigens, the properties recited for the oligopeptide (possessing positively-charged and D-amino acids) would render it difficult or even impossible to design an oligopeptide which specifically binds to the surface antigen. The specific binding of the cell surface antigen is explicitly described as an attribute of the ligand, not the oligopeptide. "A ligand can be an antibody, a fragment of an antibody, or a synthetic peptide that binds specifically to a cell surface antigen." Specification at page 5, lines 25-27. Thus, the amendment of claim 1 is supported by the linking role described in the specification for the oligopeptide and inconsistent with the specific binding role described for the ligand.

Claim 2 has been amended to specify that the one or more positively-charged amino acids referred to in the claim are D-amino acids. As discussed below, only D-amino acids satisfy the logic of claims 1 and 2 when read together. Therefore, this amendment merely specifies a condition which was logically required by the original claim, as pointed out by the examiner.

Claim 3 has been amended to clarify that the moiety of formula (II) is covalently linked to the oligopeptide through substituent X. Support is found in the specification at at page 8, lines 19-27 (emphasis added).

> The label can be a chemical moiety that is covalently attached to the oligopeptide. The chemical moiety has the structure of formula (II). For the chemical moiety in formula (II), X can be an amino group, a carboxyl group, or $(CH_2)_nSH$, wherein n is an integer from 0 to 10. . . . The chemical moiety can be a carboxylic acid

> compound attached to an amino group on the oligopeptide. Alternately, the chemical moiety can be an amino compound attached to a carboxyl group on the oligopeptide.

This is further supported atpage 10, lines 20-25.

> [A] chemical moiety of formula II can be coupled to a free amino or carboxyl group on an amino acid residue of the oligopeptide, for example to the ϵ-amino group of D-Lys. Third, a chemical moiety of formula II can be coupled to the free amino or carboxyl end of the compound of formula I.

The second passage cited reveals how the substituent X, *e.g.*, a carboxyl or amino group, can be covalently linked to available substituents on the oligopeptide, *e.g.*, a different amino or carboxyl group, including those on amino acid side chains or at the carboxyl or amino termini of the oligopeptide.

Claim 6 has been amended to recite an "interspecies" recombinant antibody rather than a "chimeric" recombinant antibody. The term "interspecies" is consistent with the terminology and teachings found in the specification and better describes what the Applicant regards as the claimed invention. Support is found at page 6, lines 8-9. "The antibody can also be a recombinant antibody, *e.g.*, a chimeric or interspecies antibody." Further support is found at page 13, lines 14-20.

> Repeated doses of murine antibodies in humans as required for optimal therapeutic efficacy lead to the development of human anti-mouse antibody responses (Tjandra et al., 1990) which may cause allergic reactions or inhibit targeting of the murine antibody to the tumor. This problem can be addressed by producing human/murine recombinant antibodies (also called humanized antibodies) which contain the tumor-specific murine variable regions linked to a human immunoglobulin constant region.

New claim 44 recites a composition of claim 1 which has two or more positively-charged amino acids. Support is found in originally-filed claim 15, which is directed to compositions of claim 1 which comprise D-Lys and D-Arg.

Objection to Claim 2 Under 37 C.F.R. 1.75(c)

Claim 2 is objected to under 37 C.F.R. 1.75 (c) as allegedly being in improper dependent form. Insufficient antecedent basis is alleged for the limitation "two or more L-amino acids, . . . separated from one another by one or more positively-charged amino acids". The parent claim allegedly precludes this situation. The parent claim recites that the oligopeptide "does not comprise two or more contiguous L-amino acids." Thus, the parent claim does not preclude the presence of two or more L-amino acids if the intervening segment comprises one or more D-amino acids, either positively charged or not. Claim 2 properly limits claim 1 by further requiring that one or more intervening D-amino acids be positively charged. Applicant therefore respectfully requests reconsideration and withdrawal of this objection.

The Rejection of Claims 2-4 and 6 Under 35 U.S.C. § 112, Second Paragraph

Claims 2, 3, 4, and 6 are rejected as indefinite. Applicant respectfully traverses the rejection.

Claim 2 is said to be indefinite because the stereochemistry of the one or more positively-charged amino acids is not defined. However, Applicant respectfully maintains that specification of the stereochemistry of those amino acids is provided by the logic of claim 2 when read in the context of claim 1, its base claim. For example, the examiner's statement that "a positively-charged D-amino acid . . . will suffice as a spacer between L-amino acids" is correct. Office Action at page 3, paragraph 6. However, the examiner's statement that a single positively-charged L-amino acid would suffice is not correct, because this condition is precluded by the recitation in the base claim that "the oligopeptide does not comprise two or more contiguous L-amino acids". In any case, the

rejection has been rendered moot by the amendment of claim 2 to recite the D stereochemistry for the one or more positively-charged amino acids. Thus, withdrawal of the rejection of claim 2 for alleged indefiniteness is respectfully requested.

Claim 3 is said to be indefinite with respect to the linkage of the moiety of formula (II) to the oligopeptide. Claim 3 has been amended to clarify that the moiety of formula (II) is linked to the oligopeptide via a covalent linkage between substituent X (which is selected from the group consisting of an amino, carboxyl, or sulfhydryl moiety) and the oligopeptide. As amended, claim 3 states clearly how the moiety of formula (II) is linked to the oligopeptide. Withdrawal of the rejection of claim 3 is respectfully requested.

The office action states no basis for the rejection of claim 4, which depends on claim 3 The alleged indefiniteness of claim 4 is presumed to carry forward from the issues related to claim 3, as discussed above. Therefore, the withdrawal of the rejection of claim 4 for alleged indefiniteness is also respectfully requested.

Claim 6 is rejected as allegedly indefinite in the recitation of a "chimeric" recombinant antibody. It is respectfully submitted that the term is not indefinite. It is defined in the specification at page 6, line 9: "The antibody can also be a recombinant antibody, *e.g.*, a chimeric or interspecies antibody produced by recombinant DNA methods." A preferred embodiment is described in the same paragraph, which refers to a humanized antibody comprising human constant regions combined with murine variable regions. Further description of chimeric antibodies used in the invention is found in the section beginning at page 13, line 13. That section teaches the use of a humanized chimeric recombinant version of the murine monoclonal antibody L8A4. The reader is further referred in that section to Hoogenboom U.S. Patent 5,565,332, which describes methods for

humanizing antibodies by chain shuffling. Thus, a chimeric antibody of the invention is described in the specification as a recombinant interspecies antibody. Accordingly, claim 6 has been amended to recite an interspecies recombinant antibody. Withdrawal of the rejection of claim 6 for alleged indefiniteness is respectfully requested.

The Rejection of Claims 1, 5, 8, 14, and 17-20 Under 35 U.S.C. § 102(a)

Claims 1, 5, 8, 14, and 17-20 are rejected as anticipated by Govindan et al. (J. Nuclear Med., Abstract, May 1998). The rejection is respectfully traversed.

Claim 1 is drawn to a composition for internally labeling a cell, comprising a ligand which specifically binds to a surface antigen of the cell, an oligopeptide which comprises at least one positively-charged amino acid residue and at least one D-amino acid residue, but which does not comprise two or more contiguous L-amino acids, and a label which is covalently bound to the oligopeptide. In claim 5 the ligand is a monoclonal antibody. In claim 8 the cell is a tumor cell. In claim 14 the oligopeptide comprises a D-Lys residue. In claims 17-20 the label comprises a radionuclide.

In order for a reference to anticipate a claimed invention, it must teach each and every recited element of the claim. *RCA Corp. v. Applied Digital Data Sys., Inc.*, 730 F.2d 1440 (Fed. Cir. 1984), *cert. dismissed sub nom. Hazeltine Corp. v. RCA Corp.*, 468 U.S. 1228 (1984). All of the subject claims recite one or more positively-charged amino acid residues in the oligopeptide. Govindan teaches iodinatable peptides comprising three or four D-amino acids and carrying a protein cross-linker at the amino terminus, which can be used to link the iodinatable peptide to a monoclonal antibody. Govindan, however, does not disclose an oligopeptide with any positively-charged amino

acids. Govindan teaches an oligopeptide which comprises lysine, normally a positively-charged amino acid by virtue of its ϵ-amino group. However, the ϵ-amino group of lysine taught by Govindan is covalently linked to DTPA, a protein crosslinking reagent. The lysine which is derivatized with DTPA as taught by Govindan does not carry a positive charge because it cannot be protonated. Therefore, since Govindan does not disclose at least one element of the subject claims (at least one positively-charged amino acid), Govindan does not anticipate the subject claims. Withdrawal of the rejection is respectfully requested.

The Rejection of Claims 1 and 21 Under 35 U.S.C. § 103(a)

Claims 1 and 21 are rejected as obvious over Kawai in view of Kindzelskii. The rejection is respectfully traversed.

Claim 1 is drawn to a composition for internally labeling a cell, comprising a ligand which specifically binds to a surface antigen of the cell, an oligopeptide which comprises at least one positively-charged amino acid residue and at least one D-amino acid residue, but which does not comprise two or more contiguous L-amino acids, and a label which is covalently bound to the oligopeptide. In claim 21 the label is fluorescent.

Kawai is cited as teaching oligopeptide ligands for the anaphylatoxin receptor. The ligands possess D-amino acids, at least one positively-charged amino acid, and no contiguous L-amino acids. Kindzelskii is cited as teaching the fluorescent labeling of oligopeptide ligands which contain D-amino acids and are internalized by neutrophils after binding to cell surface receptors. The office action states that it would have been obvious to label the anaphylotoxin receptor ligands of Kawai with a fluorescent label as taught by Kindzelskii. However, even if it were proper to combine the

oligopeptide ligand of Kawai with the labeling method of Kindzelskii, it would not lead to the subject invention. Both Kawai and Kindzelskii teach an oligopeptide ligand which specifically binds to a cell surface receptor. However, the subject claims as amended require both a ligand and an oligopeptide which does not specifically bind to the cell surface receptor. In the subject claims, specific binding to a cell surface receptor is provided by a ligand, which is an entirely distinct element of the composition. Because neither Kawai nor Kindzelskii teach or suggest a ligand and an oligopeptide as required by the subject claims, their combination cannot render the claims obvious.

Further, there is no motivation for combining the teachings of Kawai with those of Kindzelskii. Kawai teaches the synthesis of compounds which modulate anaphylatoxin activity by binding to anaphylatoxin receptors. Kawai does not teach or suggest fluorescent labeling of the anaphylatoxin ligands. Kindzelskii teaches the use of a fluorescent ligand for receptors involved in phagocytosis by neutrophils in order to study the spatial distribution of those receptors. One such receptor is the formyl peptide receptor, whose ligand is a peptide which includes D-amino acids. Kindzelskii utilizes a commercial preparation of that ligand which is conjugated to fluorescein isothiocyanate by a chemistry which is unrelated to the D or L stereochemistry of the amino acid residues of the ligand. Kindzelskii does not teach or suggest the general labeling of oligopeptides containing D-amino acids, nor the general labeling of ligands for cell surface receptors on neutrophils. Moreover, Kindzelskii does not teach or suggest the labeling of any anaphylatoxin receptor ligands, as disclosed in Kawai. Kindzelskii merely teaches the use of fluorescent ligands for receptors of phagocytosis. Therefore, there is no motivation present in either reference to suggest the combination of Kindzelskii's fluorescent labeling with Kawai's ligands.

For the reasons stated above, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 1-5, 8, 14, and 17-20 Under 35 U.S.C. § 103(a)

Claims 1-5, 8, 14, and 17-20 are rejected as obvious over Govindan in view of Zalutsky (U.S. Patent 5,302,700) or Zalutsky (CRISP Abstract). The rejection is respectfully traversed.

Claim 1 is drawn to a composition for internally labeling a cell comprising a ligand which specifically binds to a cell surface receptor and becomes internalized by the cell, an oligopeptide which comprises at least one positively-charged amino acid residue and at least one D-amino acid residue, and a label which is covalently bound to the oligopeptide. In dependent claim 2, the oligopeptide comprises two or more L-amino acids separated by one or more positively-charged D-amino acids. Claim 3 recites a type of labeling moiety, and claim 4 recites particular labels. In claim 5 the ligand is specified as a monoclonal antibody. In claim 8 the ligand is specified to bind to a tumor cell. In claim 14 the oligopeptide must comprise a D-Lys residue. Claims 17-20 recite radionuclide labels.

The office action finds all the elements of claim 1 in the teachings of Govindan, as discussed above. The Zalutsky references are said to teach two of the labels recited in claim 4 and the use of radionuclides in the label, as recited in claims 17-20. However, as previously stated, Govindan does not teach an oligopeptide comprising a positively-charged amino acid residue. Thus, the combination of Govindan and either Zalutsky reference does not lead to the claimed invention and therefore does not render the invention obvious since neither reference teaches the use of a positively-charged amino acid residue. The withdrawal of the rejection is respectfully requested.

The Rejection of Claims 1, 3-5, 8-10, 14, and 17-20 Under 35 U.S.C. § 103(a)

Claims 1, 3-5, 8-10, 14, and 17-20 are rejected as obvious over Reist in view of Govindan. The rejection is respectfully traversed.

The rejected claims are the same as for the previous rejection, with the addition of claims 9 and 10. In claim 9 the recited ligand specifically binds the receptor EGFRvIII, and in claim 10 the recited ligand is a monoclonal antibody which specifically binds EGFRvIII.

Reist teaches the labeling of a monoclonal antibody that specifically binds to EGFRvIII using radioiodine-substituted 5-iodo-3-pyridinecarboxylate. Reist also teaches that the use of a positively-charged moiety enhances resistance to lysosomal degradation and improves cellular retention of the radiolabel. Govindan is alleged to teach the use of an oligopeptide containing both D-amino acids and positively-charged D-Lys in order to link a ligand for an internalizing receptor to a label.

While Reist teaches that the positive charge of a pyridine moiety is advantageous for resisting lysosomal degradation, the only positively-charged group taught by Reist is the pyridine carboxylate group. Reist does not teach using positively-charged amino acids as recited in the claims. A positively-charged oligopeptide to link a label to a ligand is completely distinct from the positively-charged pyridine carboxylate and entails different strategies of adding the label and conjugating to the ligand. Reist simply does not teach or suggest the use of an oligopeptide for attaching the label, much less an oligopeptide comprising one or more positively-charged amino acids.

Govindan teaches the use of an oligopeptide containing D-amino acids. However,Govindan does not teach the use of any positively-charged amino acid residues because, as discussed

previously, in Govindan the ϵ-amino group of lysine is substituted with a protein conjugating agent and is not protonatable.

The office action states that it would have been obvious to combine Reist's teaching of a positively-charged pyridine carboxylate group with Govindan's teaching of an oligopeptide containing D-amino acids. However, there is no suggestion in either reference of a motivation for combining them. Reist provides no suggestion of using anything but a pyridine carboxylate group to provide positive charge. Reist does not contemplate the use of an oligopeptide to link a label to a ligand for an internalizing receptor. Neither Reist nor Govindan teach or suggest any positively-charged amino acids. Furthermore, while Reist teaches that one particular positively-charged group, a pyridine carboxylate moiety, confers some resistance to lysosomal degradation, Reist does not suggest that further positive charges beyond the pyridine group would confer any additional advantage. Govindan teaches that D-amino acids confer resistance to proteolytic cleavage, but does not teach or suggest the use of positive charge. Thus, one of ordinary skill in the art would not have been motivated to combine the teachings of Reist and Govindan to use an oligopeptide comprising one or more positively-charged amino acids and one or more D-amino acids.

Importantly, the inventor has discovered that an oligopeptide comprising both one or more D-amino acids and one or more positive charges unexpectedly improves stability to lysosomal degradation compared with either D-amino acids or positive charge alone. The degree of improvement was much greater than that observed by Reist using the positively-charged 5-iodo-3-pyridinecarboxylate moiety. This can be seen by comparing the cellular retention of label in Reist with that obtained using a positively-charged oligopeptide of the subject claims. While Reist discloses an increase in intracellular retention of up to 65% (page 4970, lines 13-15), Fig. 3 of the

subject application demonstrates that a composition employing α-*N*-Ac-D-Lys-D-Arg-D-Tyr-D-Arg-D-Arg provided a three- to four-fold (300-400%) increase in label retention after 24 hours compared to the radioiodinated antibody lacking the oligopeptide. The improvement of retention was also unexpectedly greater than that found by Govindan for D-amino acids without positive charge (reported as two- to three-fold at lines 12-14 of the Govindan abstract). This improvement is objective evidence of the non-obviousness of employing an oligopeptide comprising both one or more D-amino acids and one or more positively-charged amino acids to form a degradation-resistant linkage between a label and a ligand for an internalizing receptor.

Therefore, for the reasons discussed above, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 1, 3-5, 8-10, 14, and 17-20 Under 35 U.S.C. § 103(a)

Claims 1, 3-5, 8-10, 14, and 17-20 are rejected as allegedly obvious over Reist in view of Govindan and Emery. The rejection is respectfully traversed.

Applicant requests clarification of the status of claim 7 which is not included in this rejection. In claim 7 the ligand is a humanized antibody.

Reist teaches the labeling of a monoclonal antibody that specifically binds to EGFRvIII using radioiodine-substituted 5-iodo-3-pyridinecarboxylate. Reist also teaches that the use of a positively-charged pyridine carboxylate moiety enhances resistance to lysosomal degradation and improves cellular retention of the radiolabel. Govindan is alleged to teach the use of an oligopeptide containing both D-amino acids and positively-charged D-Lys in order to link a ligand for an internalizing receptor to a label. This rejection adds the teachings of Emery to the basis for the

previous rejection. Emery teaches the use of humanized mouse antibodies to obtain greater half-life and effectiveness with reduced hypersensitivity to mouse antibodies.

The rejection fails by the same reasoning explained above. Neither Reist, Govindan, nor Emery teach or suggest the use of one or more positively-charged amino acid residues in an oligopeptide which links a label to a ligand for an internalizing cell surface receptor. As described for the previous rejection, the invention provides a labeled composition with unexpectedly improved cellular retention compared with the combined teachings of the references. For these reasons, the withdrawal of this rejection is respectfully requested.

The Rejection of Claims 1, 5, and 8-20 Under 35 U.S.C. § 103(a)

Claims 1, 5, and 8-20 are rejected as allegedly obvious over Reist in view of Govindan and Miller. The rejection is respectfully traversed.

The subject claims are directed to a composition for internally labeling a cell, comprising a ligand which specifically binds to a cell surface receptor, an oligopeptide comprising at least one D-amino acid and at least one positively-charged amino acid residue, and a label. Some of the subject claims recite oligopeptides comprising D-Tyr (claim 11), D-Lys (claim 14), D-Arg (claim 15), or at least three D-Arg residues (claim 16). Reist teaches the labeling of a monoclonal antibody that specifically binds to EGFRvIII using radioiodine-substituted 5-iodo-3-pyridinecarboxylate. Reist also teaches that the use of a positively-charged pyridine carboxylate moiety enhances resistance to lysosomal degradation and improves cellular retention of the radiolabel. Govindan is alleged to teach the use of an oligopeptide containing both D-amino acids and positively-charged D-

Lys in order to link a ligand for an internalizing receptor to a label. Miller allegedly teaches oligopeptides comprising D-amino acids, including D-Arg, which provide resistance to proteases.

As argued against the previous two rejections, the combined teachings of Reist and Govindan do not lead to the positively-charged oligopeptide of the subject claims. Miller does not rectify this deficiency, and therefore the rejection fails.

Miller teaches oligopeptides which comprise specific target sequences for specific proteases. Miller suggests that converting an entire sequence of at least four amino acids, which is specific for a given protease, from L- to D-amino acids results in resistance to cleavage by that protease. Miller does not suggest what result would obtain if only one or a few amino acids from such a sequence were altered from L to D, nor does Miller suggest what effect such substitutions would have on sequences which do not contain specific cleavage sites for proteases. Further, Miller's teaching cannot predict resistance to lysosomal degradation in a cell, which involves multiple proteases acting simultaneously in a special environment. While Miller teaches oligopeptides comprising either one or two D-Arg residues (*see, e.g.*, Fig. 1 at page 2658), Miller's choice of Arg is arbitrary because Miller teaches the blind substitution of all D-amino acids for all L-amino acids at a protease recognition site. Miller does not teach or suggest the use of positively-charged amino acids to resist protease activity or lysosomal degradation. The teachings of Miller do not add anything beyond those of Govindan, which is cited as suggesting that D-amino acids might confer resistance to proteolytic degradation to improve label retention in a cell.

Furthermore, one of ordinary skill in the art would not be motivated to combine the teachings of Miller with those of Reist and Govindan. Miller teaches the conversion of protease recognition sites to D-amino acids. However, since the central problem addressed by both Reist and

Govindan is the prevention of proteolytic release of the label, one of skill in the art would not be inclined to use a protease recognition sequence in an oligopeptide of the invention. Therefore, there is no motivation to apply Miller to the problem addressed by Reist and Govindan.

Therefore, for the reasons cited above, the withdrawal of this rejection is respectfully requested.

Allowance of all pending claims is respectfully requested.

Respectfully submitted,

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